Product Information

Material Number: 554666
Alternate Name: CCL2; C-C motif chemokine 2; Chemokine (C-C motif) ligand 2; MCAF; SCYA2

Size: 0.1 mg
Concentration: 0.2 mg/ml
Clone: 5D3-F7
Immunogen: Recombinant Human MCP-1
Isotype: Mouse IgG1, κ
Reactivity: Tested in Development: Rhesus, Cynomolgus

Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 5D3-F7 monoclonal antibody specifically binds to human monocyte chemoattractant protein-1 (MCP-1), also known as CCL2 (C-C motif chemokine 2), Monocyte chemotactic and activating factor (MCAF), and Small-inducible cytokine A2 (SCYA2). MCP-1 is a 10-14 kDa glycoprotein member of the beta or CC family of chemokines. It is expressed by monocytes, fibroblasts, endothelial cells and other cell types in response to IL-1, IL-6, TNF, and a variety of other stimuli. MCP-1 binds to and exerts its biological activity through G-protein coupled chemokine receptors including CCR2/CD192 and CCR4/CD194. It serves as a chemoattractant and activating factor for monocytes and other cell types including T cells, NK cells, and basophils.

MCP-1 is a member of the CC chemokine family and it is produced by monocytes, T lymphocytes, fibroblasts, endothelial cells, smooth muscle cells, keratinocytes and some tumors. Its production can be induced by LPS or IFN-γ. Clone 5D3-F7 also cross reacts with an intracellular component of LPS-stimulated (24 hours) peripheral blood monocytes of rhesus and cynomolgus macaque monkeys. The staining pattern observed on non-human primate monocytes is not as strong as that seen on normal human peripheral blood monocytes.

Expression of MCP-1 by stimulated CD14+ human monocytes. Human PBMC were stimulated for 24 hours with LPS (10 ng/ml final concentration) in the presence of BD GolgiStop™ (2 µM final concentration; Cat. No. 554724). The PBMC were harvested, stained with FITC Mouse Anti-Human CD14 (Cat. No. 555397), fixed, permeabilized, and subsequently stained with 20 µl of PE Mouse Anti-Human MCP-1 (Cat. No. 559324/554666/557066) by following the Usage section below and BD Biosciences Pharmingen's staining protocol (left panel). The data reflect gating on monocytes, based on forward and side scattered light signals. To demonstrate specificity of staining, binding by PE Mouse Anti-Human MCP-1 was blocked by preincubation of the fixed/permeabilized cells with excess unlabeled Purified Mouse Anti-Human MCP-1 (5 µg; Cat. No. 551226; middle panel) prior to staining with PE Mouse Anti-Human MCP-1. The level of non-specific staining was assessed using the PE Mouse IgG1, κ Isotype Control (0.25 µg; Cat. No. 554680; right panel).

The quadrant markers for the bivariate dot plots were set based on the autofluorescence control and verified using the unlabeled antibody blocking control.
Preparation and Storage
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

**Application**

| Intracellular staining (flow cytometry) | Routinely Tested |

**Recommended Assay Procedure:**

**Immunofluorescent Staining and Flow Cytometric Analysis:** The PE Mouse Anti-Human MCP-1 (Cat. No. 559324/554666/557066) can be used for multicolor immunofluorescent staining and flow cytometric analyses to identify and enumerate MCP-1-producing cells within mixed cell populations (see image). For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/million cells). For specific methodology, please visit the protocols section under "Intracellular Flow" posted on our web site, http://www.bdbiosciences.com/us/s/resources.

A useful control for demonstrating specificity of staining is to pre-block the paraformaldehyde-fixed/saponin-permeabilized cells with Purified Mouse Anti-Human MCP-1 (Cat. No. 551226) prior to staining. The staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable mouse IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is PE Mouse IgG1, κ Isotype Control (Cat. No. 554680); use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 µg mAb/1 million cells).

**Suggested Companion Products**

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<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tr>
<td>555179</td>
<td>Human MCP-1 ELISA Set</td>
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<tr>
<td>554680</td>
<td>PE Mouse IgG1, κ Isotype Control</td>
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<td>MOPC-21</td>
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<td>Protein Transport Inhibitor (Containing Monensin)</td>
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<td>559324</td>
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**Product Notices**

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

**References**
